DIVISION OF KIDNEY, UROLOGIC AND HEMATOLOGIC DISEASES

FY 1999 Program Plan

RESEARCH PROGRESS REVIEWS

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DIVISION OF KIDNEY, UROLOGIC, AND HEMATOLOGIC DISEASES

FY 1999 PROGRAM PLAN

Research Progress Reviews

HEMATOLOGY PROGRAM

I. TITLE: Zebrafish Models for Human Blood Diseases

BACKGROUND: Sideroblastic anemias may derive from a number of causes, resulting in the presence of ringed cells in the marrow called sideroblasts, ineffective erythropoiesis, increased levels of tissue iron, and pale erythrocytes in the blood. The congenital form in humans often is caused by a mutation in delta-aminolevulinate synthase, the enzyme required for the first step in heme biosynthesis. Other heme enzyme mutations in humans cause human porphyrias, a group of rare and clinically complex syndromes. The most prevalent porphyria is porphyria cutanea tarda (PCT), which manifests skin photosensitivity and excessive excretion of uroporphyrin, due to reduced uroporphyrinogen decarboxylase (UROD). Animal models have not been available for these human disorders.

RECENT FINDINGS: The popular, small, aquarium fish called Danio rerio (or more commonly the zebrafish) is emerging as a powerful new tool to understand human genes and human development. Two recent studies highlight the success of this organism as a model to study human blood diseases. A zebrafish mutant called sauternes, because of its pale blood, has a form of anemia in which the red blood cells have a reduced volume and hemoglobin content. The gene responsible for this condition was just discovered to be the same gene that is defective in human patients with sideroblastic anemia. This gene codes for delta-aminolevulinate synthase, the enzyme required for the first step in heme synthesis. This enzyme is very similar in humans and zebrafish.

A second zebrafish mutant, yquem, has photosensitive red blood cells that are destroyed by exposure to bright light. Further study of these fish has established that they have a form of porphyria, very much like the human disease. Patients with this disease are very light-sensitive and have disordered liver and hematologic function. Fish with this mutation die when exposed to bright light; the mutation is in the same gene that is defective in humans with hepato-erythropoietic porphyria.

SIGNIFICANCE: These studies contribute to the increased awareness that many of the key proteins that determine the function of cells are widely conserved across many life forms, and that simple life forms can provide experimental models with results directly applicable to understanding human disease. Further studies in mutant fish may help identify new therapies for patients with defects in these enzymes.

FUTURE DIRECTIONS: Using these animal models, it will be possible to design protocols for gene therapy, to rescue affected animals from mutant enzymes, and cure the disease. These models may be useful for evaluating a large number of DNA constructs for gene therapy. Similar approaches may allow molecular characterization of other heme enzyme mutants in zebrafish, and the mutants should be useful in elucidating the pathogenesis of human disorders of heme biosynthesis.

ACKNOWLEDGMENTS:

Sources of Support:

Grant or Contract #	Principal Investigator	Institution
P50 DK49216	Orkin, S.A.	Boston Children's Hosp
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R01 DK54508	Lin, S.	Med College of Ga

Publication Data:

Brownlie A, Donvan A, Pratt S, Paw B, Oates A, Brugnara C, Witowska H, Sassa S, Zon L, "Positional Cloning of the Zebrafish *Sauternes* Gene: A Model for Congenital Sideroblastic Anemia," Nature Genetics 1998;20:244-50.

Wang H, Long Q, Marty S, Sassa S, Lin S, "A Zebrafish Model for Hepatoerythropoietic Porphyria," Nature Genetics 1998;20:239-43.

II. TITLE: Targeted Expansion of Genetically Modified Stem Cells

BACKGROUND: Hematopoietic stem cells are considered to be ideal target cells for gene therapy. However, their use is limited without efficient methods of transferring genes into early hematopoietic progenitors and stem cells. To overcome this obstacle, researchers have developed techniques to select and expand the numbers of genetically modified cells. The standard technique involves the transfer of a drug-resistance gene followed by exposure to the corresponding cytotoxic drug. However, the toxicity of the drug limits the use of this technique *in vivo*, and selection based on this method is difficult to apply since stem cells are resistant to most cytotoxic agents.

RECENT FINDINGS: NIDDK-funded researchers have developed a new method to select cells that results in a genetically modified cell population with a restricted and reversible growth advantage. The method uses a system that

permits intracellular protein dimerization to be reversibly activated in response to a lipid-soluble dimeric form of the drug FK506, called FK1012. FK1012 is used to bring together two FK506-binding domains from a cellular protein called FKBP12. In the experiment, FKBP12 was linked to the signaling domain of the thrombopoietin receptor, allowing interleukin-3-dependent cells to become FK1012-dependent. Dimerization of the fusion protein through the addition of FK1012 resulted in a marked proliferation of marrow cells that was restricted to the genetically modified cells. A preference for differentiation along the megakaryocyte lineage was observed.

SIGNIFICANCE: This innovative approach allows the specific delivery of a signal to divide a population of genetically modified primary cells. The technique may provide insights into blood cell development. The *in vivo* application of this approach may allow for the specific expansion of a minor population of genetically modified stem cells and progenitors, making it useful for gene therapy.

FUTURE DIRECTIONS: It needs to be demonstrated that the technique can be applied to other cell lineages, such as the erythroid and myeloid lines, through activation of the erythropoietin and GCSF receptors, respectively. Testing so far has been done only in cell lines, and must be tried in animal models.

ACKNOWLEDGMENTS:

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R01DK52997	Blau, C.A.	Univ of Washington
P30 DK47754	Miller, A.D.	Univ of Washington

Publication Data:

Jin L, Siritanaratkul N, Emery DW, Richard RE, Kaushansky K, Papayannopoulou T, Blau CA. "Targeted Expansion of Genetically Modified Stem Cells," Proceedings of the National Academy of Sciences 1998;95(14):8093-7.

III. TITLE: A More Stable and Effective Erythropoietin Molecule *in vivo*

BACKGROUND: Erythropoietin is a hormone, produced by the kidney, which

regulates the production of red cells. Its use in treating the anemia of renal failure has markedly improved the quality of life for many of those patients and for people with certain other forms of chronic anemia. Nevertheless, the drug's high cost and the need to administer it parenterally have limited its use.

RECENT FINDINGS: NIDDK-funded investigators reported recently in the *Proceedings of the National Academy of Sciences* that they have created a modified form of erythropoietin, in which two copies of the molecule are linked. The plasma half-life of erythropoietin dimers in rabbits exceeded 24 hours, compared with 4 hours for the monomers. Importantly, erythropoietin dimers were biologically active *in vivo*, as shown by their ability to raise the hematocrits of mice when injected under the skin. Finally, the dimers exhibited more than 26-fold higher activity *in vivo* than did the monomers, and were effective after only one dose.

SIGNIFICANCE: This modified molecule is more stable in the circulation and, molecule for molecule, much more effective. This advance raises the possibility of improved and more cost-effective treatment of a variety of forms of anemia.

FUTURE DIRECTIONS: Studies are needed to test this modified erythropoietin in people. The approach may be generally applicable to other therapeutic proteins, including but not limited to hematopoietic growth factors and coagulation factors.

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Grant and Contract #	Principal Investigator	Institution
R01DK38841	Sytkowski, A.J.	Harvard Medical School

Publication Data:

Sytkowski AJ, Lunn ED, Davis KL, Feldman L; Siekman S, "Human Erythropoietin Dimers with Markedly Enhanced *in vivo* Activity," Proceedings of the National Academy of Sciences 1998;Feb 3;95(3):1184-8.

IV. TITLE: Potential Safety Issue With Widely Studied Chelator, Deferiprone (L1)

BACKGROUND: Patients with beta-thalassemia (Cooley's Anemia) continue to

suffer from the sequelae of transfusion-induced iron overload due to the inadequacies of current iron-chelation therapy. Compliance with the use of subcutaneous desferioxamine continues to be a major problem, despite convincing evidence that it markedly reduces morbidity and prolongs life. The full potential of iron-chelation therapy will not be realized until an orally-effective drug is available.

One widely studied oral candidate, deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one, L1), until recently has been considered by many to represent the most likely to be selected for clinical use.

RECENT FINDINGS: A long-term study in thalassemia patients at the Toronto Hospital for Sick Children identified a potential problem with the iron-chelator deferiprone. Of 14 patients given the drug, 5 developed progressive hepatic fibrosis. This liver complication was found in none of the patients who received deferoxamine. The authors raised questions about the safety and efficacy of long-term deferiprone therapy. However, an accompanying editorial urged further analysis, since iron overload itself causes hepatic fibrosis. The editorial also raised the possibility of sampling errors in the study and pointed out differences between deferiprone-treated patients in whom the fibrosis worsened and those in whom it did not.

SIGNIFICANCE: These results indicate that deferiprone may be associated with worsening of hepatic fibrosis, even in patients whose hepatic iron concentrations have stabilized or declined.

FUTURE DIRECTIONS: Prospective clinical trials are needed to evaluate the possibility of irreversible hepatic damage from L1. Improvements in the administration of iron chelators may prove useful, as in a study now being supported by the NIDDK involving a combination of two oral iron chelators, deferiprone and HBED, in a small number of patients with complete metabolic studies. Based on preliminary patient studies, there appears to be synergistic iron excretion when these chelators are used in combination. The search for additional new chelators needs to continue.

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Grant or Contract #	Principal Investigator	Institution
R01DK49108	Bergeron, R.J.	University of Florida

Publication Data:

Olivieri N, Brittenham G, McLaren C, Templeton D, Cameron R, McClelland R, Burt A, Fleming K, "Long-Term Safety and Effectiveness of Iron-Chelation Therapy with Deferiprone for Thalassemia Major," New England Journal of Medicine 1998;339(7):417-23.

V. TITLE: Identification of a Novel Cofactor, Friend of GATA-1 (FOG), Regulating Erythroid Development

BACKGROUND: GATA transcription factors have emerged as central regulators of diverse developmental processes in both vertebrate and invertebrate species. A hematopoietic-restricted transcription factor, GATA-1, is expressed at high levels in hematopoietic cells. Fundamental to the understanding of how GATA-1 functions is the elucidation of the mechanisms by which it alters transcription of genetic information.

RECENT FINDINGS: NIDDK-funded researchers have identified a novel, multitype zinc-finger protein, FOG, which now has been demonstrated to be required *in vivo* as a GATA-1 cofactor in erythroid cells. FOG also has been established as a pivotal, GATA-1-independent factor in the earliest stages of megakaryocyte development.

SIGNIFICANCE: It is apparent that the activity of GATA transcription factors is modulated by interactions with FOG, and possibly with certain other related proteins. Loss of FOG leads to the specific ablation of the megakaryocytic lineage, pointing to an absolute requirement for FOG during early platelet development. These findings have important implications for the role of FOG and other FOG-like proteins in regulating transcription and development. FOG and GATA-1 have been established as components of an essential protein complex in erythroid cells.

FUTURE DIRECTIONS: The possibility exists that the FOG/GATA-1 complex may contain additional proteins, which need to be identified and characterized. FOG also may act as a cofactor for other hematopoietic GATA factors. Defining how FOG functions in early megakaryocytopoiesis is likely to reveal novel mechanisms by which FOG and related proteins regulate transcription and development.

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P50 DK49216	Orkin, S.A.	Boston Children's Hospital

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Tsang P, Fujiwara Y, Hom D, Orkin S, "Failure of Megakaryopoiesis and Arrested Erythropoiesis in Mice Lacking the Gata-1 Transcriptional Cofactor FOG," Genes and Development 1998;12(8):1176-88.

UROLOGY PROGRAM

VI. TITLE: The First Oral Drug is Approved for Male Erectile Dysfunction

BACKGROUND: Erectile dysfunction, also called impotence, affects an estimated 30 million men, including up to 70 percent of men with diabetes. The development of non-surgical treatments for erectile dysfunction has accelerated rapidly since NIDDK's 1992 Consensus Development Conference on erectile dysfunction. The conference established a clinical definition of erectile dysfunction and increased public awareness about the extent of the problem. Treatments have become sequentially less invasive, moving from prostheses to penile injections to drugs inserted into the penis. However, there was no FDA-approved oral treatment. Earlier NIDDK-funded studies had shown that erection occurs when smooth muscles in the corpora cavernosa relax following a chemical reaction in which nitric oxide from cavernous nerves triggers guanylate cyclase to form cyclic guanosine monophosphate (cGMP). The recently approved oral drug sildenafil inhibits cGMP-specific phosphodiesterase type 5, the isozyme that metabolizes cGMP in the corpora cavernosa.

RECENT FINDINGS: Sildenafil's inhibition of cGMP-specific phosphodiesterase type 5 would be expected to restore the natural erectile response to sexual stimulation, but not without such stimulation. Recent results from two short-term, double-blind clinical efficacy studies in men with erectile impotence demonstrated that 69 percent of all attempted sexual intercourse was successful in men receiving sildenafil, compared to only 22 percent in men taking a placebo. Men in the study had erectile dysfunction of organic, psychogenic or mixed causes, including diabetes, hypertension, and ischemic heart disease, and following radical prostatectomy. Sildenafil increased erectile function but did not change sexual desire.

SIGNIFICANCE: This is the first reported double-blind, clinical study to demonstrate the effectiveness of an oral drug for erectile dysfunction. Sildenafil opens a new era for the effective treatment of male erectile dysfunction and for research.

FUTURE DIRECTIONS: Sildenafil is an important tool for treating impotence, but not all men respond to the drug. Both basic and clinical research can increase both the effectiveness and number of available treatments, and identify treatments that can be targeted at specific causes of erectile dysfunction. Understanding how cGMP relaxes the cavernosal smooth muscle and how diseases such as diabetes cause erectile dysfunction will help accomplish these

goals. In addition, knowing how sildenafil and other oral agents in development work in men with different causes of impotence will contribute to our ability to rationally choose treatments for individual patients.

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Grant or Contract #	Principal Investigator	Institution
R01DK39080	Goldstein, I.	Boston University
R01DK40025	Traish, A.	Boston University
R29DK45087	Azadzoi, K.M.	Boston University

Publication Data:

Goldstein I, Lue TF, Padma-Nathan H, Rosen RC, Steers WD, Wicker PA. "Oral Sildenafil in the Treatment of Erectile Dysfunction," New England Journal of Medicine 1998;338:1397-1404.

VII. TITLE: Molecular Evidence of Stem Cell Compartments in the Prostate

BACKGROUND: Understanding cell growth in the prostate is important to help explain the development of benign prostatic hyperplasia (BPH). In several types of rapidly renewing tissues, stem cells, transiently proliferating cells, and mature terminally differentiated cells occupy discrete locations and often form stratified layers. The prostatic epithelium has two major compartments, basal and secretory. Most epithelial or secretory cells in the adult prostate are androgen-dependent. Although the cell layers of the adult prostate renew more slowly than layers in other organs, a similar stem-cell-driven hierarchical arrangement has been postulated. However, there has been little molecular evidence of a transiently proliferating cell compartment in the prostate, and the location and nature of the stem cells still is unknown.

RECENT FINDINGS: NIDDK-funded researchers have identified and characterized cells in the human prostate capable of entering the proliferating phase of the cell cycle. The investigators used differential expression of the cyclin-dependent kinase inhibitor p27Kip1 to demonstrate distinct cell populations in the normal prostate. A cell layer that was consistently p27Kip1-negative was identified between the basal and secretory layers. Cells in this middle layer were accentuated in the periurethral (BPH forming) layer and in

tissue subjected to androgen blockade. These cells are likely to be the population that undergoes abnormal growth in BPH.

SIGNIFICANCE: The identification of this transiently proliferating cell compartment demonstrates for the first time a method to identify the existence, location and interrelationship between the three significant cell layers (stem cell; transiently proliferating; and mature, terminally differentiated) in the adult prostate.

FUTURE DIRECTIONS: Identification of specific layers involved in cell development will allow gene therapy to be targeted to those layers to influence the growth of adult prostate tissues.

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Grant or Contract #	Principal Investigator	Institution
R01DK22000	Coffey, D.S.	Johns Hopkins Univ
P01DK19300	Coffey, D.S.	Johns Hopkins Univ

Publication Data:

DeMarzo AM, Meeker AK, Epstein JI, Coffey DS, "Prostate Stem Cell Compartments. Expression of the Cell Cycle Inhibitor $p27^{kip1}$ in Normal, Hyperplastic, and Neoplastic Cells," American Journal of Pathology 1998;153 (3):911-9.

VIII. TITLE: Insights into Genetic Factors Involved in Kidney Stones

BACKGROUND: Kidney stones affect many adults. The most prevalent type of stone is made of calcium oxalate. Although stone disease has been reported since the age of Hippocrates, and the chemistry of kidney stones has been extensively studied, little is known about the genetic mechanisms predisposing to stone formation. Oxalic acid (oxalate) is made in the liver, where it has no known function, and excreted by the kidneys. Excessive production can cause a number of problems, including kidney stones, nephrocalcinosis and systemic oxalosis. The latter condition occurs in people with primary hyperoxaluria, an inherited disorder in which hepatic enzyme deficiencies promote excessive oxalate production. Despite the clinical significance of calcium oxalate, many features of the biosynthetic pathway have not been determined, and there are no

therapies to inhibit oxalate production by the liver.

RECENT FINDINGS: Investigators funded by NIDDK have now defined the terminal steps of the oxalate synthetic pathway and determined that oxalate synthesis is modulated by the metabolic state of the liver and the resultant changes in NAD:NADH ratios, and lactate and alanine levels.

- Glycolate is the most important source of glyoxylate, which is then catalyzed to oxalate by the enzyme glycolate oxidase (GO). The major enzymes associated with the terminal steps of these conversions are in peroxisomes. NADH was shown to be a potent inhibitor of oxalate production. These findings have lead to development of drugs that inhibit specific enzymes such as GO, which may be useful in decreasing oxalate synthesis and urinary oxalate excretion.

- A phase I clinical study to evaluate the safety and pharmacokinetics of one such enzyme inhibitor, (L)-2-oxothizolidine-4-carboxylate (OTZ), demonstrated decreased urinary oxalate excretion in healthy men.

- Another investigator has described a gene and gene product responsible for an inherited form of hypercalciuric nephrolithiasis (X-linked hypercalciuric nephrolithiasis). Using positional cloning, the gene, CLCN5, was identified as a member of the CLC family of voltage-gated chloride channels. Abnormalities in thick ascending limb function might explain defective renal tubular calcium reabsorption, and the clinical findings of nephrolithiasis and nephrocalcinosis evident in persons with this disorder.

SIGNIFICANCE: These findings provide significant insights into the genetic basis for a predisposition to develop calcium oxalate stones. They also present preliminary data on a novel enzymatic inhibitor that may reduce oxalate synthesis in the liver. From these findings, additional treatments might be developed based on mechanisms to alter cellular NADH levels. These strategies provide a therapeutic option not only for those with the rare and devastating primary hyperoxaluria, but also possibly for people with idiopathic calcium oxalate stones.

FUTURE DIRECTIONS: Basic animal studies designed to characterize the oxalate metabolic pathway in the liver, as well as identification of specific gene products will enable the development of more targeted therapies for all forms of calcium oxalate stone disease.

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Grant or Contract #	Principal Investigator	Institution
R01DK50644	Holmes, R.P.	Bowman Gray Sch Med
R01DK46838	Scheinman, S.J.	SUNY Hlth. Ctr. Syracuse

Publication Data:

Poore RE, Hurst CH, Assimos DG, Holmes RP, "Pathways of Hepatic Oxalate Synthesis and their Regulation," American Journal of Physiology 1997;272 (Cell Physiology 41):C289-94.

Holmes RP, Assimos DG, Leaf CD, Whalen JJ, "The Effects of (L)-2-oxothiazolidine-4-carboxylate on Urinary Oxalate Excretion," Journal of Urology 1997;158:34-7.

Hoopes RR, Hueber PA, Reid RJ, Braiden GL, Goodyer PR, Melnyk AR, Midgley JP, Moel DI, Neu AM, VanWhy SK, Scheinman SJ, "CLCN5 Chloride-Channel Mutations in Six New North American Families with X-Linked Nephrolithiasis," Kidney International 1998;54:698-705.

Scheinman SJ, "X-Linked Hypercalciuric Nephrolithiasis: Clinical Syndromes and Chloride Channel Mutations," Kidney International 1998;53:3-17.

IX. TITLE: Recurrent Urinary Infections in Women: Contributing Molecular and Bacteriological Factors

BACKGROUND: Women with recurrent urinary tract infections (UTIs) often have persistent vaginal colonization with *E. coli*. Evidence suggests that alterations of the normal, *Lactobacillus*-dominant vaginal flora may predispose to colonization with *E. coli. In vitro* studies have demonstrated that strains of hydrogen peroxide-producing *lactobacilli* inhibit the growth of *E. coli*. Similarly, it has been previously demonstrated that women with recurrent UTIs are significantly more likely to be nonsecretors of blood group antigens than are women without such a history. The vaginal epithelial cells from these nonsecretors (women with recurrent UTIs) enhance adherence of uropathogenic *E. coli* isolates more often than the secretors.

RECENT FINDINGS: Recently published clinical data from women with and without recurrent UTIs demonstrates that women without recurrent UTIs were significantly more likely to have vaginal colonization with these H_2O_2-forming

lactobacilli, and that spermicides were associated with increased vaginal *E. coli* colonization and absence of the H_2O_2-forming *lactobacilli*. The same investigators studied women with recurrent *E. coli* UTIs, and separated them into secretors and non-secretors of blood group antigens. They have now demonstrated two specific glycosphingolipids (GSLs) of the vaginal epithelial cells (VEC) from these women. One of these, sialosyl galactosyl globoside (SGG), was expressed in human kidney tissue. SSG selectively binds certain uropathogenic *E. coli*. Researchers concluded that SGG expression plays an important role in the pathogenesis of UTIs.

SIGNIFICANCE: These studies suggest that the total microbial ecology of the vagina influences *E. coli* colonization, and that repletion of H_2O_2-forming *lactobacilli* could reduce the risk of recurrent UTI. Avoiding factors such as spermicides that decrease the concentration of H_2O_2-forming *lactobacilli* could also effectively reduce UTI recurrence. In addition, novel strategies to prevent UTIs may be developed using carbohydrate-based compounds that competitively inhibit bacterial attachment.

FUTURE DIRECTIONS: The mechanisms whereby H_2O_2-forming *lactobacilli* prevent colonization of the vagina with uropathogens needs to be further examined and characterized. Further studies are needed to define the expression of SGG in epithelial tissues throughout the urogenital tract. Data from these bladder tissue studies will increase our knowledge of bladder glycobiology.

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R01DK40045	Stamm, W.E.	University of Wash
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Gupta K, Stapelton AE, Hooton TM, Roberts PL, Fennell CL, Stamm WE, "Inverse Association of H_2O_2-Producing *Lactobacilli* and Vaginal *Escherichia coli* Colonization in Women with Recurrent Urinary Tract Infections," Journal of Infectious Diseases 1998;178:446-50.

Stapleton AE, Stroud MR, Hakomori SI, Stamm WE, "The Globoseries Glycosphingolipid Sialosyl Galactosyl Globoside is Found in Urinary Tract

Tissues and is a Preffered Binding Receptor *in vitro* for Uropathogenic *Escherichia coli* Expressing *pap*-Encoded Adhesins," Infection and Immunity 1998;66:3856-61.

X. TITLE: Novel Use of Knockout Mice and Genetic Engineering in Bladder Injury and Repair

BACKGROUND: The factors that influence bladder growth, development, and response to injury are not well delineated. Response to bladder injury involves cellular proliferation, migration, and differentiation; removal of damaged tissue; and production of extracellular matrix. Many of these actions also occur during bladder development and growth, and all may be controlled by growth factors. Studies have shown that epidermal growth factor (EGF) is expressed in bladder urothelium, and this family of peptides may mediate urothelial growth. A related substance in skin, keratinocyte growth factor (KGF), is induced after an injury.

RECENT FINDINGS: The role of growth factors in bladder pathophysiology has been an area of intense investigation. Whole bladders transplanted from EGF knockout mice into adult nude rats have normal bladder regeneration after injury. Such findings suggest the EGF pathway is not necessary for bladder regeneration. On the other hand, KGF has a direct effect on urothelial proliferation. In addition, both KGF and transforming growth factor " (TGF") are involved in bladder wound healing and have direct effects on urothelial proliferation. NIDDK-funded researchers have also reported that bladder urothelial cells can be transfected with various genes, and then transferred to a biodegradable polymer scaffold from which a neo-genetically engineered organ develops.

SIGNIFICANCE: These findings provide significant new pathways for the development of novel, organ specific therapies for bladder reconstruction after resection for disease. For example, it is possible that a bladder could be constructed *de novo* to replace a diabetic neuropathic bladder. A tissue-engineering approach might allow transfected cells to be confined and localized, and ultimately removed, if there is evidence of mutagenesis or immunogenicity. Tissue could also be engineered to express specific growth factors which have been demonstrated to be effective in urothelial growth, differentiation and repair.

FUTURE DIRECTIONS: These studies are opening an entirely new concept--engineering urothelial cells--to treat bladder diseases and disorders.

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R01DK49484	Atala, A.	Boston Children's Hospital
R01DK51397	Cuha, G.	Univ of Calif-SF

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DiSandro MJ, Baskin LS, Li YW, Werb Z, Cunha GR, "Development and Regenerative Ability of Bladder in the Transgenic Epidermal Growth Factor Receptor Gene Knockout Mouse," Journal of Urology 1997;158:1058-65.

Baskin LS, Sutherland RS, Thomson AA, Nguyen HT, Morgan DM, Hayward SW, DiSandro M, Cunha GR, "Growth Factors in Bladder Wound Healing," Journal of Urology 1997;157:2388-95.

Yoo JJ, Atala A, "A Novel Gene Delivery System Using Urothelial Tissue Engineered Neo-Organs," Journal of Urology 1997;158:1066-70.

XI. TITLE: Risk Factors for Urinary Tract Infections (UTI)

BACKGROUND: UTIs are common, especially in women. Controversy over the association between UTIs, sexual activity, and spermicides remains.

RECENT FINDINGS: A large study in HMO patients identified sexually active young women with acute UTIs and compared them to population-based control patients from the same HMO. Sexual activity and contraceptive practices were identified during interviews. Younger age, intercourse frequency, prior UTI, and frequency of use of spermicide-coated condoms were independent predictors of *staphylococcus saprophyticus* infection, the second most common cause of UTI in young women. These investigators previously demonstrated that spermicide-coated condoms were associated with 41 percent of UTIs from *E. coli*, the leading cause of UTIs in young women.

SIGNIFICANCE: This study identifies correlates of UTI in a population of young women receiving care in an HMO setting and identifies factors that may be remediable.

FUTURE DIRECTIONS: Changing behaviors regarding use of spermicides may

decrease the incidence of UTIs in susceptible women.

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RO1DK41341	Fihn, S.	Univ of Wash

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Fihn SD, Boyko EJ, Chen C-L, Normand EH, Yarbro P, Scholes D, "Use of Spermicide-Coated Condoms and Other Risk Factors for Urinary Tract Infection Caused by *Staphylococcus Saprophyticus*," Archives of Internal Medicine 1998;158:281-7.

KIDNEY PROGRAMS

Renal Epidemiology Program

XII. TITLE: Barriers to Renal Transplantation

BACKGROUND: There is a serious shortage of organs for transplantation, and questions about equitable distribution have arisen.

RECENT FINDINGS: Two NIDDK-funded studies assessed the odds of transplantation for women and African Americans compared to men and Caucasians. The researchers found that female gender and African American race are barriers to transplantation, not only in trying to get on a waiting list but also in obtaining a transplant once on a list. The researchers suggest that these hurdles are unrelated to the separate issue of matching donor organs to recipients.

Because women had more difficulty getting on waiting lists and receiving transplants once there, they were 25 percent less likely to be transplanted compared to men, according to one study. A second study defined four stages to transplantation: 1) being medically suitable and willing to consider transplantation; 2) being definitely interested in transplantation; 3) completing the pre-transplant work-up; and 4) being on a waiting list and receiving a transplant. At each stage, women were less likely than men to complete the stage, and African Americans were less likely to complete stages two through four.

SIGNIFICANCE AND FUTURE DIRECTIONS: These studies will help increase awareness about access to kidney transplantation and support efforts to ensure equitable access regardless of gender or race.

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R01DK51472	Seghal, A.R.	Case Western Res Univ
RO1DK49531	Bloembergen, W.E.	University of Michigan

Publication Data:

Alexander GC, Sehgal AR, "Barriers to Cadaveric Renal Transplantation Among

Blacks, Women, and the Poor," Journal of the American Medical Association 1998;280:1148-52.

Bloembergen WE, Mauger EA, Wolfe RA, Port FK, "Association of Gender and Access to Cadaveric Renal Transplantation," American Journal of Kidney Disease 1997;30:733-8.

XIII. TITLE: Renal Vascular Disease in Different Ethnic Groups

BACKGROUND: Previous studies reported that renal vascular disease was uncommon in African Americans, suggesting a low need to screen this population for the disease.

RECENT FINDINGS: Using duplex sonography, researchers examined the kidneys of people in the Forsyth County group of the Cardiovascular Health Study. Surprisingly, the authors found significant renal vascular disease in 11.8 percent of hypertensive African Americans, a rate comparable to that expected in the general population. After surgery for the vascular disease, kidney function improved in many of these patients, especially among those with poorer renal function.

SIGNIFICANCE AND FUTURE DIRECTIONS: This study suggests a need to screen for renal vascular disease in African Americans, especially those who have high blood pressure. Further efforts must be made to study outcomes in different populations after screening for renal vascular disease and instituting various therapeutic plans.

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R01DK47414	Hansen, K.	Bowman Gray Sch of Med

Publication Data:

Hansen KJ, Deitch JS, Dean RH, "Renovascular Disease in Blacks: Prevalence and Result of Operative Management," American Journal of the Medical Sciences 1998;315:337-42.

XIV. TITLE: High Mortality Among Dialysis Patients After Heart Attack

BACKGROUND: Cardiovascular disease is the most common cause of death in dialysis patients in the United States. Outcomes of dialysis patients after myocardial infarction have not been studied before.

RECENT FINDINGS: Patients who were hospitalized between 1977 and 1995 for a first heart attack were identified by the investigators using NIDDK's U.S. Renal Data System. Overall mortality after a heart attack was 59.3 percent at 1 year and 89.9 percent after 5 years. More recent enrollees had higher overall death rates from all causes, probably because they had a greater number of other health problems at the start of ESRD therapy.

SIGNIFICANCE: These studies have helped to identify poor outcomes in the ESRD population that may be remediable.

FUTURE DIRECTIONS: Efforts to improve cardiovascular outcomes in end-stage renal disease (ESRD) patients, regardless of other health problems, should be undertaken. Investigating outcomes for ESRD patients with other illnesses may lead to improved outcomes for all patients.

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Herzog CA, Ma JZ, Collins AJ, "Poor Long-Term Survival After Acute Myocardial Infarction Among Patients on Long-Term Dialysis," New England Journal of Medicine 1998;339:799-805.

Renal Transplantation Program

XV. TITLE: Tolerance to Non-Inherited Maternal HLA Antigens and Survival of Renal Transplants

BACKGROUND: Kidney transplantation is the most important and physiologic renal replacement therapy. However, only a small proportion of patients with ESRD are able to receive a kidney transplant, and many transplanted kidneys do not survive. The 90-day graft survival is 91 percent, and 1 and 2-year graft survival is 87 and 79 percent, respectively, according to NIDDK's U.S. Renal Data System. These relatively short-term survival probabilities have shown remarkable improvement over the past decade, but long-term survival has not. In 1986, 10-year graft survival was only 33 percent.

Most failed transplants have been attributed to chronic rejection. The T cells of the recipient seem to play the critical role in the rejection process by recognizing histocompatibility antigens from the donor. Although immunosuppressive drugs help in suppressing the T cell response to these foreign antigens, naturally induced tolerance of the recipient to the foreign antigens should help with improved graft survival. During pregnancy, exposure of the fetus to maternal cells and antigens induces tolerance, in some cases, to non-inherited maternal histocompatibility antigens. Theoretically, therefore, transplants between siblings would show improved survival if the differences in HLA antigens were limited to the non-inherited maternal histocompatibility antigens.

RECENT FINDINGS: In this NIDDK-funded study, investigators analyzed data on graft survival in 205 people who received a first kidney transplant from a living-related sibling mismatched for one HLA haplotype. The appropriate immuno-suppressive regimen was used in all patients. The results show that when a patient receives a kidney from a sibling with maternal HLA antigens he/she did not inherit, the long-term graft survival is improved. The 10-year graft survival (where such data is available) in such transplants was 77 percent, which was not significantly different from the 10-year graft survival between HLA-identical siblings during the identical period.

SIGNIFICANCE: This study should help in selecting sibling pairs for transplantation. To enhance graft survival, transplants should be encouraged between siblings who differ only at the non-inherited maternal HLA antigen locus whenever possible.

FUTURE DIRECTIONS: Other databases should be analyzed to confirm the observation made in this study, as well as to identify other HLA mismatches that are not particularly disadvantageous. Conceivably, such observations will extend similar “beneficial mismatches” in cadaveric renal transplantation.

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Chronic Renal Diseases and Pediatric Nephrology Program

XVI. TITLE: Pathogenesis of Polycystic Kidney Disease

BACKGROUND: Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder affecting an estimated 1 in 400 to 1 in 1000 people. ADPKD consists of at least three genetically distinct disorders characterized by bilateral renal cysts and progressive enlargement of the kidneys. About 50 percent of disease-gene carriers progress to kidney failure. Mutations in the PKD1 gene on chromosome 16 account for about 95 percent of clinically recognized cases, while the remainder of cases are largely from mutations in PKD2 on chromosome 4, the second gene for ADPKD. Autosomal recessive polycystic kidney disease (ARPKD) constitutes a very serious renal disease in children, often leading to ESRD in childhood. The ARPKD gene has not been cloned.

Dramatic progress has been made over the past 2 to 3 years, regarding the etiology, pathogenesis and clinical course of PKD. Research continues to focus on the genetics, mechanisms of cyst formation and growth, identification of risk factors for susceptibility to ESRD, and the development of animal models.

RECENT FINDINGS: Somatic inactivation of PKD2 results in PKD. Germline mutations in PKD2 cause ADPKD. A mutant exon 1, in tandem with the wild-type exon 1, was introduced at the mouse PKD2 locus. This unstable allele undergoes somatic inactivation by intragenic homologous recombination to produce a true null allele. Mice heterozygous and homozygous for this mutation develop PKD and liver lesions indistinguishable from the human phenotype. These studies establish that somatic loss of PKD2 expression is both necessary and sufficient for renal cyst formation in ADPKD, suggesting that PKD2 occurs

by a cellular recessive mechanism.

- Gene conversion, identified as a mechanism of mutation for a number of human genes, is a likely cause of mutation in PKD1. About 70 percent of the gene responsible for ADPK1 is replicated in several highly homologous copies located nearby on chromosome 16. A novel strategy for finding mutations in the duplicated region of ADPK1 was recently described. The technique uses one gene-specific primer from PKD1 as an anchor, in combination with a primer from the duplicated portion to amplify ADPKD1-specific templates. Using changes in restriction digest patterns, investigators showed that sequence substitutions were present in unrelated patients with a nearly identical cluster of base pair substitutions involving exon 23. This was also shown in a rodent-human somatic cell hybrid that contains only PKD1 homologues. These changes were also detected in total DNA from several affected and unaffected individuals who did not harbor this mutation in their PKD1 gene copy. This is the first example of gene conversion in PKD1. These findings highlight the importance of using reagents proven to be locus-specific in identifying and defining *PKD1* mutations that may reflect sequence differences also present in homologous loci.

- Researchers recently reported identifying PKDL, a novel PKD2-like gene whose murine homologue is detected in mice with kidney and retinal defects. Polycystin-1 and B2 are the products of PKD1 and PKD2 genes that are mutated in most cases of ADPKD. It has been suggested that polycystin-2 may function as a subunit of an ion channel regulated by polycystin-1. Investigators report the identification of the homologous human PKDL gene, which encodes a new member of the polycystin protein family called polycystin-L. The full-length transcript of PKDL is expressed at high levels in fetal tissues, including kidney and liver, and down-regulated in adult tissues. PKDL may be an excellent candidate for as-yet-unmapped cystic diseases in people and animals.

- Previous data indicated that renal cyst development in PKD1 is likely to require somatic inactivation of the normal allele coupled to a germ-line PKD1 mutation. The same group of investigators used unique reagents and reported that intragenic somatic mutations are common in liver cysts. All pathogenic mutations altered the previously normal copy of the gene. These new data extend the “two-hit” model of cyst development to include a second focal manifestation of the disease.

- PKD1 mutations on chromosome 16p13.3 account for 85 to 95 percent of all PKD cases. The gene has an unusual bipartite structure, with 70

percent of its 5ʾ end duplicated in other places on chromosome 16. Another important feature of the human PKD1 gene is a cluster of two long polypyrimidine tracts in adjacent introns in the center of the gene. Polypyrimidine tracts can form triple helices with diverse biological effects, affecting gene expression and enhancing mutagenesis in a variety of ways. To test the hypothesis that the differences in the genomic structure of the murine and human PKD1 genes might be responsible for the different rates of mutation observed, these polypyrimidine tracts were sought in the mouse. The hybridization and sequence data from these studies clearly show that the mPKD1 *(*murin*e* PKD1) does not contain the polypyrimidine tracts found in the human gene. A previous study also reported that the mouse genome, in contrast to that of humans, has only one copy of PKD1. An important question is whether the PKD1 homologues or the pyrimidine tracts play a role in the pathogenesis of human ADPKD. Differences in the genomic structure of murine and human PKD1 genes may be responsible for their different mutation rates. The PKD1 homologues in humans are likely to account for at least some of the increased mutability of human PKD1, since having more than one copy of the gene can promote mutation. Polypyrimidine tracts in human PKD1 may significantly contribute to the gene's overall instability. Additional studies are attempting to further characterize the role of these structures, including their presence and/or absence in regulating the gene's activity and mutability in humans.

SIGNIFICANCE: New opportunities in genetics, as a result of advances in cellular and molecular biology, and selected clinical and animal studies should help elucidate the etiology and pathogenesis of PKD and the related cellular and molecular mechanisms that determine kidney structure and function in general.

FUTURE DIRECTIONS: Work should focus on using pathogenic principles to design and test specific therapies in animal models, with the prospect of ultimate relevance in human disease.

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XVII. TITLE: Renal Disease Progression

BACKGROUND: Renal fibrosis resulting from progressive extracellular matrix accumulation is a main cause of chronic renal disease and the putative final common pathway of renal injury in animals and humans. Fibrosis of the glomerulus and the tubulointerstitium impairs kidney function and ultimately leads to organ failure. Mechanical factors and other mediators, including cytokines, growth factors, and eicosanoids derived from circulating or glomerular cells, have been implicated in initiating or maintaining sclerosis. Considerable evidence has accumulated showing that overproduction of the cytokine transforming growth factor β (TGF-β) plays a key role in the development of renal fibrosis. Diverse actions of TGF-β on collagen turnover may be either immediate or mediated by synthesis of regulatory molecules.

A possible explanation for the kidney's particular susceptibility to fibrosis in response to injury may be the recent discovery of biologically complex interactions between the renin-angiotensin-aldosterone system (RAAS) and certain cytokines and other biologic systems. RAAS has broad impact on progression of sclerotic vascular diseases, by both hemodynamic and non-hemodynamic mechanisms.

RECENT FINDINGS: New information about the interaction of RAAS with cytokines and other factors is emerging regarding powerful effector molecules that preserve systemic and tissue homeostasis. Of particular relevance is the role and interaction of RAAS and TGF-β in the kidney and the molecular mechanisms involved.

- Angiotensin II (Ang II) infusion strongly stimulates the production of TGF-β in the kidney, and Ang II-blockade reduces TGF-β over-expression in kidney and heart. TGF-β has been shown to be a powerful fibrogenic cytokine which acts to simultaneously stimulate the synthesis of extracellular matrix, to inhibit the actions of proteases that degrade matrix, and to increase the expression of cell surface integrins that interact with matrix components.

- In addition, the role of the plasmin protease system in turnover of the mesangial matrix was recently shown. Plasmin has been long recognized as a fibrinolytic enzyme, important in dissolving clots after injury. This molecule, like Ang II and TGF-β, is rapidly increased at the site of a wound, where it stabilizes the fibrin clot, serving as scaffolding for platelet aggregation and temporary matrix production. A relatively new role for this system has been characterized as highly relevant to fibrotic renal diseases. In addition to degrading fibrin, plasmin acts on a

wide range of extracellular matrix proteins, cleaving some procollagenases to produce active molecules that degrade collagens.

- Aldosterone overproduction has been linked to hypertension and glomerulo-sclerosis. These data suggest that aldosterone may have fibrogenic effects, independent of Ang II.

- Finally, an ex-vivo gene transfer system to deliver cytokines into the kidney and circulation was constructed, using genetically modified renal tubular epithelial cells (TEC) infected with recombinant retroviruses expressing macrophage growth factors. These TECs are capable of secreting stable, sustained amounts of cytokines for long periods *in vitro* and *in vivo*. Implanting these TEC-secreting macrophage growth factors under the kidney capsule initiates severe local renal injury in a murine model. This system offers a novel and powerful approach to probe for the impact of sustained cytokine expression in progression of kidney disease.

SIGNIFICANCE: Fibrotic diseases are characterized by the accumulation of extracellular matrix, or scar tissue. A better understanding of the intricate mechanisms leading to matrix accumulation and scarring in the glomeruli and tubulointerstitium should help identify strategies to ultimately control progression of chronic renal diseases.

FUTURE DIRECTIONS: Studies are needed to develop experimental models of progressive renal disease to further characterize the biological events involved in glomerular and tubulointerstitial extracellular matrix accumulation leading to renal scarring; to define biological interactions between the RAAS and the fibrinolytic system, and the impact of common genetic polymorphisms in angiotensin converting enzyme (ACE) and plasmin on the progression of renal disease; to identify and test strategies to influence expression of factors whose actions/interactions have a central role in mediating renal fibrosis.

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R01DK43609	Border, W.A.	University of Utah
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XVIII. TITLE: The Immune Basis of Lupus Nephritis

BACKGROUND: Systemic Lupus Erythematosus (SLE) is a chronic disease with a broad spectrum of life-threatening complications, affecting mostly women of childbearing ages. Most people with SLE have some degree of renal disease, and many have kidney failure. The etiology remains unknown. However a consistent manifestation of immune dysfunction is the hyperactivity of the immune system, both *in vitro* and *in vivo*. Family, twin and ethnic studies suggest a genetic component in the pathogenesis of SLE. Patients with specific HLA genes and genes that encode for antigen-presenting proteins are associated with differential susceptibility to developing autoimmune disease.

RECENT FINDINGS: Autoantibodies (auto-Ab) target a diverse group of tissue antigens in human and experimental autoimmune kidney disease. Subpopulations of auto-Ab produce different disease profiles with distinguishable histologic and clinical patterns. The initial event and the site are determined by the location of the autoantigens and the direct interactions with the auto-Ab.

- A unique subset of anti-DNA-Ab that entered cells and localized within the nuclei *in vivo* was identified and studied. Following the administration of anti-DNA Ab fragments into normal mice, these immunoglobulins were detected within the cell nuclei of various organs. In the kidney, this was associated with hypercellularity and protein in the urine. The abnormalities appeared to be mediated by a direct effect of the intranuclear antibodies within the glomerular cells. Nuclear localization depended on the unique antigenic binding properties of a subset of lupus auto-Ab. Cellular entry was initiated by the binding of the nuclear-localizing anti-DNA Ab to the myosin-1 cell surface receptor. These data provide insight into the behavior of Ab within the cytoplasm en route to the nucleus. Evidence from other laboratories suggests that myosin 1 forms a DNase-1 and calmodulin complex, potentially interfering with intracellular enzyme activity.

- Another NIDDK-funded laboratory indicates that lupus-like auto-Ab are readily generated in subjects of normal genetic background by random recombination absent mutations. Auto-Ab may contribute to disease if normal immune system regulation is disturbed. In a transgenic murine model, experimental findings supported the hypothesis that autoreactive B cells capable of producing pathogenic immunoglobulins are generated *in vivo* in normal individuals and may contribute to disease if normal immunoregulation is disturbed.

- In the MRL-*Fas lpr* murine model of human SLE, renal disease progresses rapidly, leading to predictable 50 percent mortality at 6 months of age, and is regulated by a single gene mutation. Renal injury involves glomerular, perivascular, and interstitial pathology, and is mediated by macrophages, T cells, and cytokines. Nephropathy is determined by the MRL+/+ background genes, interacting with a single gene mutation in *Fas. T*he MRL genes are responsible for autoimmune kidney disease and the *Fas lpr (Fas* deficiency) mutation converts a latent, mild nephritis into a rapid, fulminant disease. Colony stimulating factor (CSF)-1 and tumor necrosis factor (TNF)-α are cytokines implicated in developing renal injury. CSF-1 is responsible for eliciting autoimmune kidney destruction and is expressed in the kidneys before disease-development, increasing with advancing kidney damage. Gene transfer of CSF-1 into MRL-*Fas lpr* kidneys elicits renal injury. Gene transfer of TNF-α fails to elicit autoimmune kidney injury; dual gene transfer of TNF-α and CSF-1 amplifies the renal damage produced by CSF-1 alone. Similarly, injecting TNF-α fails to incite renal injury, but accelerates pathology in mice with nephritis.

- T cells are required for autoimmune kidney disease in this model, since during renal injury these cells infiltrate the glomeruli, interstitium, and perivascular compartments, secreting interferon (IFN)-γ. Blockade of IFN-γ signaling prevents glomerulonephritis and prolongs survival. An IFN-γR-deficient strain was constructed to ascertain whether IFN-γ is responsible for cytokine-, macrophage-, and T-cell-dependent kidney damage, and whether IFN-γ is responsible for programmed (apoptotic) cell death, directly or indirectly. IFN-γ was required for cytokine production and renal parenchymal cell apoptosis. The MRL-*Fas lpr* mice lacking the IFN-γR are protected from fatal lupus nephritis.

- Using a retroviral gene transfer strategy, researchers established an association between renal expression of RANTES (a β-chemokine, chemoattractant for macrophages and T cells) and renal inflammatory injury. Tubular epithelial cells, genetically modified to secrete RANTES and infused under the renal capsule, incited interstitial nephritis in MRL-*Fas lp* mice. In addition, delivery of RANTES and CSF-1 into the kidney of this murine model caused an additive increase in pathology.

SIGNIFICANCE: The observations identifying a previously unrecognized means of cellular entry and transit of proteins into and within cellular compartments should lead to a better understanding of the events underlying renal injury in patients with SLE. It should be possible to take advantage of this information to design carrier proteins for transit and targeting of other molecules to the

nucleus, interfering with injury. This approach should lead to potential fruitful therapeutic applications.

FUTURE DIRECTIONS: Because cytokines influence immunity and inflammation, interventions that modify cytokine pathways or destroy cytokine receptor-bearing cells may be effective for modulating harmful inflammatory responses. Further studies with murine antibodies or antagonist molecules that block nephro-pathogenic substances would be highly desirable. Strategies that interfere with the expression of such deleterious molecules and their targets could lead to promising therapeutic interventions and prevention of autoimmune renal injury.

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XIX. TITLE: Pathogenesis of Glomerulonephritis/Tubulointerstitial Nephritis

BACKGROUND: Immune mechanisms are the predominant cause of most forms of glomerulonephritis (GN) and tubulointerstitial nephritis (TIN). Research on GN and TIN has benefited greatly because of advances in cellular and molecular biology and the ability to create and test genetic mutations in animal models. The new tools of molecular biology have been very useful to this field of research, and the emphasis on transgenic and knock-out models producing genetic mutations continues to enhance the study and testing of experimental therapies in preparation for future human research.

RECENT FINDINGS:

- Endogenous fibroblast growth factor-2 (FGF-2) is released from mesangial cells in experimental mesangioproliferative GN. Anti-FGF-2 therapy led to significant reductions in glomerular injury secondary to expression of α-smooth muscle actin, mesangial cell proliferation, matrix accumulation, and platelet influx. Conversely, injections of FGF-2 augmented glomerular inflammation. Studies of mechanisms underlying the amplification of cellular injury by FGF-2 showed that anti-FGF-2 therapy considerably reduced cell death after disease induction. These data suggest that release of constitutively expressed FGF-2 after immune-mediated cell injury contributes to glomerular cell damage and implicates FGF-2 as a novel mediator of toxicity.

- Many forms of human glomerular disease are autoimmune in nature,

characterized by glomerular deposits of immunoglobulin and complement. A significant protective effect of CD59 (cell membrane-bound complement regulatory protein on glomerular cells), which inhibits C5b-9 (membrane attack complex) assembly and insertion, was demonstrated in a new rat model of immune thrombotic microangiopathy. These data confirm that C5b-9 formation has a critical pathogenic role in the mediation of the disease. CD59 may be important in protecting the glomerular endothelium from other complement-mediated injuries.

- NIDDK-funded investigators have characterized the cellular events that occur in the glomeruli and tubulointerstitium in a complement-independent murine nephritis model, characterized by glomerular crescents, progressive glomerulosclerosis and tubulointerstitial fibrosis. Early crescent formation in this model appeared to be due to proliferation of intrinsic glomerular (parietal or visceral) cells and was associated with local platelet-derived growth factor (PDGF) receptor expression. Despite the local expression of osteopontin, a potent monocyte chemoattracting and adhesive factor, neither infiltrating macrophages nor T cells could be identified before Bowman's capsule ruptured.

- The role of osteopontin expression was investigated in a rat model of accelerated anti-GBM GN. Osteopontin was expressed with one of its ligands, CD44, in intrinsic renal cells, and its expression was associated with signs of progressive nephropathy. *De novo* osteopontin mRNA expression was evident in glomerular visceral and parietal epithelial cells; preceded the development of hypercellularity, focal and segmental lesions, and crescent formation; and correlated with macrophage infiltration. Up-regulation of osteopontin by tubular epithelial cells also preceded and correlated with interstitial macrophage and T-cell infiltration.

- Tubulointerstitial fibrosis is one of the most important histologic features predicting progression of kidney disease. Thrombospondin 1 (TSP1) is an extracellular matrix protein that activates latent TGF-β. NIDDK-funded researchers examined the expression of TSP1 in several animal models of GN-associated tubulointerstitial disease. TSP1 mRNA and protein were transiently increased in tubular cells, myofibroblasts and macrophages in areas of tubulointerstitial injury; its expression always preceded the development of fibrosis and correlated quantitatively and spacially with the development of interstitial fibrosis. TSP1 expression also predicted the severity of tubulointerstitial fibrosis better than the degree of macrophage or myofibroblast accumulation, or increased TGF-β1 expression. These data are consistent with the possibility that TSP1

may be an endogenous activator of TGF-β.

- Cyclosporin A (CsA) nephropathy is associated with a marked increase in apoptosis of tubular and interstitial cells, mediated in part by Ang II and nitric oxide inhibition, suggesting a role for renal ischemia in this process. CsA-induced apoptosis correlated with interstitial fibrosis. The increase in apoptosis along with the increased production of growth factors such as TGF-β1 and PDGF may act in concert to promote tubulointerstitial fibrosis. Accelerated apoptosis could account for the host's inability to effectively remodel tissue, thereby playing a role in the pathogenesis of fibrosis and chronic transplant rejection.

SIGNIFICANCE: Many advances in understanding disease mechanisms may lead to the development of new therapies for humans, including cytokine and growth factor antagonists and receptor blockers, and agents that suppress the effects of oxidants and proteases produced by both circulating and glomerular cells. Common to these areas is the enormous potential of modern technology to continue to provide new insights into mechanisms of glomerular disease and to generate new techniques and reagents for the study and treatment of disease in humans.

FUTURE DIRECTIONS: Studies using specific peptides to block TGF-β1 activation by TSP1 and other mediators may prove important. Studies are needed to determine how the proposed pathway of ischemia and apoptosis can be interrupted to prevent the toxic effects of CsA and possibly other nephrotoxins. Research on renal inflammatory processes should focus on the development of transgenic models, structural protein chemistry, cloning biologic mediators, and establishing renal cell lines in culture. Special efforts should be focused on gene therapy and establishing a national consortium to treat patients with GN and TIN.

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Renal Cell Biology/physiology Program

XX. TITLE: Physiologic Roles of Sodium/Hydrogen Exchangers in Health and Disease

BACKGROUND: The proximal tubule reabsorbs more than 60 percent of the sodium chloride (NaCl) and water filtered by the glomerulus. Defining the molecular mechanisms of Na+ reabsorption in this nephron segment is therefore of great importance for understanding disease states resulting from abnormal renal NaCl homeostasis, such as hypertension and edematous disorders. In the proximal tubule, the most important apical membrane pathway for Na^+ reabsorption is sodium/hydrogen (Na^+/H^+) exchange.

Na^+/H^+ exchangers mediate the electroneutral exchange of Na^+ and H^+ across

plasma membranes of all cells in the body. Inhibited by amiloride and its analogs, Na^+/H^+ exchangers participate in diverse cellular functions, including intracellular pH regulation, transepithelial ion transport, cell volume regulation and cellular responses to mitogens and growth factors. Molecular cloning studies by the laboratories of Shull and Donowitz identified five distinct isoforms of Na^+/H^+ exchangers expressed in mammalian tissues (NHE1-5). A major focus of research during recent years has been to identify the sites of expression, physiologic roles, and mechanisms of regulation of NHE isoforms along the nephron in general, and in the proximal tubule in particular.

RECENT FINDINGS: By using isoform-specific antibodies, considerable progress has been made in defining the sites of expression of NHE isoforms along the nephron.

- Aronson found that the relatively ubiquitous "housekeeping" isoform NHE1 is expressed on the basolateral membrane of tubular epithelial cells throughout the nephron. In contrast, the epithelial-specific isoform NHE3 was localized to the brush border membrane of proximal tubule cells, as well as the apical membrane of cells in the loop of Henle and was found to be a basolateral isoform in renal tubular cells. Studies have also begun to define the topology of NHE3 with respect to the membrane bilayer.

- The physiological roles of NHE isoforms have been most thoroughly studied in the proximal tubule. Studies by Aronson made use of known differences in inhibitor sensitivity among NHE isoforms to demonstrate that virtually all Na^+/H^+ exchange in the brush border membrane of proximal tubule cells is mediated by NHE3. A novel approach has been the use of knockout mice with completely deficient expression of NHE isoforms. Mice with a homozygous null mutation in NHE1 were found to have no detectable effect on plasma electrolytes or acid-base status, consistent with its basolateral expression. In contrast, NHE3 null mice developed a profound defect in Na^+ and HCO3 reabsorption in the proximal tubule. The use of mice with deficient expression of individual NHE isoforms will be an important tool for understanding the physiologic roles of NHE isoforms in more detail.

- The efforts of many investigators have focused on defining the mechanisms for regulation of NHE3 in the proximal tubule. Indeed, there is new evidence that acute pressure natriuresis causes redistribution of NHE3 from the apical membrane to an intracellular membrane compartment in proximal tubule cells. Studies have also defined the important role of nonreceptor tyrosine kinases in regulating NHE3 activity.

SIGNIFICANCE: There has been enormous recent progress in defining the molecular mechanisms mediating and regulating Na^+ transport in the proximal tubule. These studies enhance our ability to understand the pathophysiology of hypertension and edematous disorders, and provide novel insight into new therapeutic targets for management of these major clinical problems.

FUTURE DIRECTIONS: An important future direction is to develop a more complete understanding of the molecular mechanisms regulating NHE3 activity in the proximal tubule and other nephron segments. Given emerging evidence that variants in genes governing renal NaCl homeostasis predispose to hypertension and fluid and electrolyte disorders such as Liddle, Bartter, and Gitelman's syndromes, an important future direction will be to test whether variants in NHE3 or its regulatory proteins cause similar clinical disorders. Transgenic and knockout mice will serve as important models for clinical disorders resulting from mutations in genes encoding NHE isoforms or regulatory proteins.

ACKNOWLEDGMENTS:

Sources of Support:

Grant or Contract #	Principal Investigator	Institution
R37DK33793	Aronson, P.S.	Yale University
R01DK19407	Warnock, D.G.	Univ of Alabama
R01DK53161	Warnock, D.G.	Univ of Alabama
R37DK39298	Alpern, R.J.	Univ of Tx-Southwestern
R01DK50599-03	Shull, G.E.	Univ of Cincinnati
R01DK34316-13	McDonough, A.A.	Univ of Southern Calif

Publication Data:

Biemesderfer D, Rutherford PA, Nagy T, Pizzonia JH, Abu-Alfa AK, Aronson PS, "Monoclonal Antibodies for High Resolution Localization of NHE3 in Adult and Neonatal Rat Kidney," American Journal of Physiology 1997;273:F289-99.

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XXI. TITLE: Role of Aquaporins in Kidney Water Transport and as Carriers for Gases Across Cell Membranes

BACKGROUND: Most molecules that cross cell membranes move through special membrane proteins that act as channels or transporters. Until recently it was generally believed that gases such as carbon dioxide (CO_2) and such small uncharged molecules as water (H_20) and urea did not need such special carrier proteins, and instead moved freely through the lipid bilayer. However, this issue is being extensively reappraised. The transport proteins for water and urea are

particularly active areas of investigation in our kidney disease program. The existence of a specialized protein named aquaporin-1 (AQP-1) that mediates water movement in red cells was first discovered in 1992. Subsequently seven members of the AQP family have been identified. Studies of these proteins are providing information about their functions and distribution, as well as the mechanisms regulating their activities. Little has been known about the permeability of these channels to small molecules other than water. The role of aquaporins in kidney water transport has also been unclear. AQP-1 was also found to be heavily expressed at sites of rapid water transport in the kidney (proximal tubule and descending limb of Henle's loop), but the initial reports suggested that its absence did not change kidney function.

RECENT FINDINGS: Recent results by an NIDDK grantee indicate that the over-expression of AQP-1 significantly increases the permeability of $C0_2$, and are consistent with the possibility that AQP-1 may act as a gas channel. These experiments were performed in frog eggs provided with an enzyme carbonic anhydrase, which results in acidification of the cell interior when $C0_2$ enters the egg. The investigators measured $C0_2$ permeability using pH-sensitive microelectrodes to measure the initial rate at which $C0_2$ entry decreased intracellular pH. The increase was reversed by a mercurial compound that blocks water movement through AQP-1.

Other related studies examine the regulation of water transport in kidneys of mice made congenitally deficient in AQP-1. The animals were found to have markedly altered kidney function and enhanced susceptibility to dehydration.

SIGNIFICANCE: Membrane transport proteins are very important for many biological functions, since they are critical to maintaining the cell interior. These proteins are important candidates for drug development, and understanding the relationship between their three-dimensional structure and their small molecule permeability may help in the development of new drugs to protect renal and vascular cells from stress and to protect red cells in the hypertonic kidney medulla. In addition, understanding the biological roles of membrane proteins in gas permeability may open new possibilities to understand disease processes.

FUTURE DIRECTIONS: Early studies of patients lacking AQP-1 did not uncover any kidney defects, but the methods used were insensitive, and this issue will need reappraisal in view of the mouse studies. Gas permeation through protein channels is an understudied topic, and these studies raise the possibility that channels may also be important in movement of another biologically important gas, nitric oxide.

ACKNOWLEDGMENTS:

Sources of Support:

Grant or Contract #	Principal Investigator	Institution
P01DK17433	Boron, W.F., Giebisch, G.H.	Yale University
R01DK40163	Sly, W.S.	St. Louis University
R01DK43955	Zeidel, M.L.	Univ of Pittsburgh

Publication Data:

Nakhoul NL, Davis BA, Romero MF, Boron WF, "Effect of Expressing the Water Channel Aquaporin-1 on the $C0_2$ Permeability of *Xenopus* oocytes" American Journal of Physiology 1998;274 (2 Pt 1):C543-8.

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XXII. TITLE: Functional Roles of ROMK Channels in Renal Handling of Potassium

BACKGROUND: Stability of body potassium balance requires the kidney to match the amounts excreted in the urine to those that are in the diet. Two developments have contributed significantly to understanding renal potassium homeostasis. First, the introduction of patch-clamp techniques has permitted characterization and elucidation of the functional behavior of renal potassium (K^+) channels in apical and basolateral membranes of defined tubule segments of the nephron. Second, the recent cloning of several K^+ channels led to progress in establishing important relationships between molecular structure and function of renal K^+ channels.

New insights into the mechanism of potassium secretion during the past 4 years have been provided by cloning of several potassium channels from renal tissue. The first of these to be cloned was the Inwardedly Rectifying K^+ channel (K_{IR})

(rectification means changing conductance with voltage), a prototype of which is the renal K^+ channel or ROMK 1 (K_{IR} 1). This was the necessary breakthrough that has led to isolating a family of related channels,

including ATP-sensitive K^+ channels, and muscarinic receptor-activated K^+ channels.

RECENT FINDINGS: Recent studies clarify the mechanisms that regulate potassium channel activity. Regulation is achieved by protein kinase A-induced phosphorylation on the kidney's ROMK channels, an effect that depends upon a newly described anchoring protein. Antenatal Bartter syndrome is a variant of an inherited renal tubular disorder associated with hypokalemic alkalosis beginning in the developing fetus. The syndrome is due to a defect in this potassium channel, which leads to a deficit in thick ascending limb (TAL)-Na-K-2Cl cotransport activity because luminal potassium is lacking. Marked hypercalciuria and, as a secondary consequence, the development of nephrocalcinosis and osteopenia are characteristics of this variant. Recent reports demonstrate 14 novel mutations in people with antenatal Bartter syndrome. Also, this channel appears to be unusual in requiring normal interaction with an unrelated but interesting class of membrane proteins, the ABC transporters. Tools are now available to examine the regulation of these proteins that can affect primary mechanisms of ion transport.

SIGNIFICANCE: Potassium balance is critical for maintenance of life. Recently, potassium loss has been implicated as critical in the deaths of several young athletes using diuretic drugs to lose weight. Disordered potassium balance may also contribute to the increased myocardial instability seen in people taking diuretics for hypertension. Understanding potassium channel regulatory mechanisms may lead to improved strategies for developing newer diuretic drugs. Since the ROMK1 channel has been shown to be mutated in some patients with Bartter syndrome, a condition of sodium and potassium wasting, future studies should also provide insight into the pathology of this syndrome.

FUTURE DIRECTIONS: The challenge of future studies in the area of K^+ secretion will be to characterize further the behavior of K^+ channels in physiological and pathophysiological conditions, to investigate the interaction of messengers with the channel proteins, and to elucidate the way channels are synthesized and targeted to specific membrane sites. We anticipate that the development of ROMK-deficient mice will also provide insights into the function of these molecules.

ACKNOWLEDGMENTS:

Sources of Support:

Grant or Contract #	Principal Investigator	Institution
R01DK37605	Hebert, S.C.	Vanderbilt University
R29DK47402	Wang, W.	N Y Medical College
P01DK17433	Giebisch, G.H.	Yale University
R01DK27847	Palmer, L.G.	Cornell University

Publication Data:

Ali S, Chen X, Xu JZ, Hebert SC, Wang WH, "The A Kinase Anchoring Protein is Required for Mediating the Effect of Protein Kinase A on ROMK Channels," Proceedings of the National Academy of Sciences 1998;95(17):10274-8.

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XXIII. TITLE: Role of Cytochrome P-450s in Hypertension

BACKGROUND: About 50 million adults in the United States suffer from high blood pressure, a major risk factor for cardiovascular, cerebrovascular and renal diseases. Hypertension and its complications are major contributors to the national budget for health care. Although the cause of essential hypertension is unknown, data from segregation and twin studies suggest genetic factors could account for an estimated 45 percent of interracial differences in blood pressure. The study of renal mechanisms that control fluid volume and composition, the characterization of the molecular basis of hypertension, and the identification of relevant candidate genes are of great interest and importance.

A role for the microsomal P-450 in the metabolism of arachidonic acid (AA) was first shown in 1981. P-450-derived AA metabolites modulate renal Na^+ and water transport, are vasoactive, and are regulated by dietary salt intake. P-450-derived AA metabolites are involved in the onset of hypertension in a rat model of genetically controlled spontaneous hypertension (the SHR/WKY model). These observations provide functional and biochemical bases for a role of the

renal P450 AA epoxygenase and ω/ω-1 hydroxylase in the pathophysiology of experimental spontaneous and salt-sensitive hypertension.

RECENT FINDINGS:

- Three research avenues have been applied to study the mechanism of action of the products of the AA epoxygenase (EETs) and ω/ω-1 hydroxylases (19- and 20-OH-AA): 1) administration of synthetic, metabolically stable, eicosanoid analogs; 2) cell transfection studies using cDNA coding for selective AA epoxygenase or ω/ω-1 hydroxylases; and 3) the development of murine strains containing disrupted P450 genes. Importantly, mice carrying mutated P-450 4a14 alleles do not synthesize P450 4a14 and are hypertensive (mean arterial blood pressures of 105±10; 140± 20; and 155±20 mm of Hg, for the (+/+), (+/-) and (-/-) genotypes, respectively). This study found that a blood pressure differential of approximately 50 mm of Hg is associated with a single gene deletion. This important finding: 1) confirms many of the proposed renal roles for the P-450 AA monooxygenase; 2) establishes a solid link between blood pressure and a unique P-450 gene; and 3) underlines the physiological and/or pathophysiological importance of this enzyme system.

- Studies by an NIDDK grantee demonstrated that $CoCl_2$-treatment of rats both attenuates the inhibition of proximal tubule Na^+ reabsorption and diuresis and abolishes Na,K-ATPase inhibition and NHE3 redistribution during acute hypertension, thereby providing further evidence that these responses may be mediated by cytochrome P-450 arachidonate metabolites.

- Growth factors such as epidermal growth factor (EGF), synthesized in the kidney, are highly concentrated in urine and have been implicated in various aspects of renal function, including recovery from acute renal injury, hypertrophy, and inflammation. In addition to their effects on renal ion transport and circulation, the mitogenic properties of the 14,15 form of epoxyeicosatrienoic acid (EET) have been documented in several renal cell lines. However, the mechanisms underlying EET-induced mitogenesis remained unclear. Administration of the sulfonimide analog of 14,15-EET to cultured renal epithelial cells results in activation of a tyrosine kinase phosphorylation cascade, rapid increases in DNA synthesis, and cell proliferation. Furthermore, cell transfection studies using cDNA coding for a region- and stereoselective 14(S),15(R) AA epoxygenase demonstrated unequivocally that the epoxygenase pathway

plays a role in mediating EGF signaling and mitogenesis. These EGF-receptor-dependent EET actions involve phospholipase A_2 activation, P450 oxidative metabolism of AA, and the activation of the PI-3 kinase and MAPK cascades. The sulfonimide analog of 11,12-EET was used to demonstrate that the renal hemodynamic effects of this EET are associated with a protein kinase A-mediated vasodilation of the afferent arteriole.

SIGNIFICANCE: With the exception of a few rare diseases affecting ion channel activity or aldosterone biosynthesis, these studies of the 4a14 knockout mouse provide one of the first demonstrations that high blood pressure can result from alterations in a single gene. Perhaps the most important implication of these observations is that they provide strong evidence that a similar genetic component could be responsible for a yet-to-be-defined subset of human hypertension. The presence in humans of P450 4A isoforms has been demonstrated, and two human 4A isoforms have been cloned (P-450s 4A9 and 4A11). Initial characterization shows that these enzymes are the human homologues of rat 4A and murine 4a P450 isoforms. Inasmuch as the human P450s 4A are also functional homologues of rat and mouse P450s 4A, these results point to a potential role for these genes in the pathophysiology of human hypertension, and suggest them to be strong candidate genes for hypertension in the human population. Identification of the genetic factors responsible will provide a better understanding of the molecular basis of human hypertension and lead to improved diagnosis, risk-assessment, and treatment.

FUTURE DIRECTIONS: Gene-targeted methods need to be developed that will provide a mouse strain containing a disrupted P-450 4a14 gene. Future research needs to address: 1) the use of recombinant adenovirus vectors for studies of EGF-dependent, epoxygenase-mediated cyto-protection; 2) the characterization of the roles played by dietary salt and animal age in the hypertensive phenotype of 4a14 (-/-) mutant mice; 3) hemodynamic and tubular effects resulting from deletion of the 4a14 gene and their relationship to blood pressure regulation; 4) the identification, cloning, and enzymatic character-ization of the human homologues of rat P-450 4A2 and mouse P-450 4a14; and 5) the genomic structure of the human P-450 4A gene subfamily.

ACKNOWLEDGMENTS:

Sources of Support:

Grant or Contract #	Principal Investigator	Institution
P01DK38226	Capdevila, J.	Vanderbilt University

Publication Data:

Helvig C, Dishman E, Capdevila JH, "Molecular, Enzymatic, and Regulatory Characterization of Rat Kidney Cytochrome P-450 4A2 and 4A3," Biochemistry 1998;37:12546-58.

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XXIV. TITLE: Altered Renal Responses in Pregnancy

BACKGROUND: Normal pregnancy is associated with water retention and dilution of the plasma. The mechanisms of altered water metabolism have yet to be fully elucidated. The fact that the collecting duct water channel aquaporin 2 (AQP2) plays a pivotal role in renal water regulation is the basis for the hypothesis that AQP2 expression could be modified during pregnancy. Preeclampsia is a poorly understood disorder in pregnancy associated with edema, hypertension, and proteinuria. Alterations in renal function play a prominent role in this disorder.

RECENT FINDINGS: NIDDK-funded researchers have demonstrated that upregulation of AQP2 in the papilla contributes to water retention in pregnancy. The expression of AQP2 mRNA early in pregnancy, and AQP2 protein was also increased. Plasma vasopressin concentrations in pregnant rats were no different from those of non-pregnant rats. When a V2 vasopressin antagonist was administered, expression of AQP2 mRNA was suppressed. These results indicate that AQP2 expression could indeed be modified during pregnancy.

SIGNIFICANCE: The results of these studies indicate that upregulation of AQP2

contributes to water retention in pregnancy through a V2 receptor-mediated effect. These data may eventually lead to insights into the treatment of preeclampsia.

FUTURE DIRECTIONS: To better understand the physiology and pathophysiology of preeclampsia, future studies are needed to examine whether nitric oxide synthase (NOS) isoforms are involved in the hemodynamic, neurohumoral and sodium and water retention of pregnancy. Approaches will include the development of knockout mice in which vasoactive or vasoconstrictor systems have been modified, by either disrupting a gene responsible for the synthesis of a vasoactive peptide or coding for a receptor of a vasoactive peptide.

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Grant or Contract #	Principal Investigator	Institution
P01DK19928	Schrier, R.W.	Univ of Colorado

Publication Data:

Ohara M, Martin P, XU D, St. John J, Pattison TA, Kim JK, Schrier RW, "Upregulation of Aquaporin 2 Water Channel Expression in Pregnant Rats," Journal of Clinical Investigation 1998;101:1076-83.

Diabetic Nephropathy Program

XXV. TITLE: Evidence of Pancreas Transplantation Providing Long-Term Benefits for the Kidney

BACKGROUND: Much evidence suggests that the kidney disease of diabetes is related to long-term control of high blood sugar. Some data have also suggested that renal damage may actually be reversed if blood sugar is tightly controlled.

RECENT FINDINGS: Pancreas transplantation, when successful, results in long-term correction of hyperglycemia. NIDDK-supported research published in the *New England Journal of Medicine* demonstrated in patients that after 10 years of normal blood sugar following pancreas transplantation, glomerular and tubular basement membrane thickness and mesangial and mesangial matrix-

fractional volume returned toward normal levels.

SIGNIFICANCE: These data highlight the beneficial effects of successful pancreas transplantation in people with type 1 diabetes mellitus and early kidney disease. A normal blood glucose level is an important goal to protect the kidney, and reversal of damage is possible. Practically, it should be noted, the beneficial effects of pancreatic transplantation on kidney disease must be balanced against the toxic effects of the current immunosuppressants, the risks of surgery, and the adverse consequences of lifelong immunosuppression.

FUTURE DIRECTIONS: Work is needed to increase blood sugar control in people with diabetes and to increase access to pancreas transplantation for those who have the kidney disease of diabetes.

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Grant or Contract #	Principal Investigator	Institution
R01DK13083	Mauer, M.	Univ of Minn Sch of Med
RO1DK43605	Mauer, M.	Univ of Minn Sch of Med

Publication Data:

Fioretto P, Steffes MW, Sutherland DER, Goetz FC, Mauer M, "Reversal of Lesions of Diabetic Nephropathy after Pancreas Transplantation," New England Journal of Medicine 1998;339:69-75.

XXVI. TITLE: Insights into Therapy and Hyperglycemic Injury To Renal Cells

BACKGROUND: High blood sugar, or hyperglycemia, is associated with renal injury. Various angiotensin converting enzyme-inhibiting (ACEI) drugs have been implicated in improved outcome in patients with diabetic and non-diabetic renal disease. The action of angiotensin II on renal cells has been implicated in pathologic processes, including increased matrix synthesis, thought to be central to the pathogenesis of diabetic nephropathy. A role for transforming growth factor-β1 (TGF-β1), a mediator of increased matrix synthesis, has been suggested in the kidney disease of diabetes.

RECENT FINDINGS: NIDDK-funded researchers have demonstrated that

increased concentrations of D-glucose in murine mesangial cell cultures were associated with increased TGF-β1 synthesis, showing increased TGF-β1 supernatant bioactivity and increased transcription of TGF-β1 mRNA in cells exposed to high concentrations of glucose. A possible glucose-responsive element in the TGF-β1 promoter was identified.

NIDDK-supported scientists have also demonstrated that both enalapril (an ACEI) and losartan, an angiotensin II receptor antagonist, decreased levels of TGF-β1, fibronectin, and plasminogen activator inhibitor-1 (PAI-1) in treated rat models of glomerulonephritis compared to untreated animals. In addition, the expression of these injury mediators in the treated animals was also reduced, as was glomerular histologic damage.

SIGNIFICANCE: These data show ambient glucose concentration is enough to cause maladaptive cellular reactions that can be associated with the development of diabetic kidney disease, and that available medications that inhibit the renin-angiotensin axis are active at the cellular level to blunt such responses.

FUTURE DIRECTIONS: Studies are needed to establish downstream events associated with TGF-β activation and molecular mechanisms involved in TGF-β inhibition using drugs inhibiting angiotensin at the tissue level. The results of ongoing trials of angiotensin receptor antagonists in people with kidney disease of diabetes will be of great interest.

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Grant or Contract #	Principal Investigator	Institution
R01DK44513	Ziyadeh, F.N.	Univ of Penn
R01DK45191	Ziyadeh, F.N.	Univ of Penn
R01DK49342	Noble, N.A.	Univ of Utah
R01DK43609	Border, W.A.	Univ of Utah
R01DK49374	Border, W.A.	Univ of Utah

Publication Data:

Hoffman BB, Sharma K, Zhu Y, Ziyadeh FN, "Transcriptional Activation of Transforming Growth Factor-β1 in Mesangial Cell Culture by High Glucose Concentration," Kidney International 1998;54:1107-16.

Peters H, Border WA, Noble NA, "Targeting TGF-β1 Overexpression in Renal Disease: Maximizing the Antifibrotic Action of Angiotensin II Blockade," Kidney International 1998;54:1570-80.

NIDDK MINORITY TRAINING AND CAREER DEVELOPMENT-1998

Name of Program and Description	Division	# of NIDDK Awards	NIDDK Funding Level	ORMH Collab. Funding
Minority Access to Research Careers (MARC) T-34) NIDDK Co-funds with NIGMS. Funds predoctoral faculty fellowships, visiting scientists, conferences for minority investigators and minority health issues, and honors undergraduate training in biomedical research. Summer Internship Program in the NIDDK Division of Intramural Research (students-managed by NIDDK-EEO).	DK-wide	6	$23,236	
Minority Biomedical Research Support Program (MBRS) NIDDK co-funds with NIGMS. Provides expanded opportunities for minorities to participate in biomedical research careers. Supports research projects of interest to the NIDDK at Minority and Equal Opportunity Institutions.	DK-wide	25	$1,985,728	
R-13 (Conference Grant) to the American Physiological Society, FASEB Provides support for underrepresented minority students to attend meetings of the Society, and for 36 minority high school science teachers to have summer research training in laboratories of Society members.	DK-wide	1	$74,315	

Initiatives for Underrepresented Minorities in Biomedical Research NIH-wide program initiatives to support minority undergraduate, graduate students, high school students, and faculty members on NIDDK active research grants through administrative supplements.	DK-wide	120	$4,500,000	
Research Training of Underrepresented Minorities on Institutional Training Grants (T32) Highly qualified Minority Investigators are assigned T-32 slots held in reserve for this purpose. DDEMD=5 DDDN=3 DKUHD=6	DK-wide	14	$175,174 81,437 183,000	$41,917
Pre-doctoral Fellowships (F-31) To provide support to minority students for research training leading to M.D.-Ph.D. in the biomedical sciences. DDEMD=6 DDDN=2 DKUHD=1	DK-wide	9	$132,269	
Cell/Molecular Biology Student/Teacher Learning Center (R-25) Laboratory Research experience for minorities in the District of Columbia (managed by NIDDK-EEO).	DK-wide	1	$334,767	

<u>Small Research Grants (R-03) for Minority Researchers</u> DDEMD=5 DDDN=n/a DKUHD=1 ORMH Collaboration provides additional support for minority researchers.	 DK-wide ORMH	 6	 $367,229 84,750	 $466,933
<u>Minority High School Student Summer Research Training Supplement</u> In conjunction with the National Minority Organ Tissue Transplant Program award to Howard University, NIDDK provides meaningful laboratory research experience to minority high school students to stimulate their interest in careers in biomedical science.	 DK-wide	 1	 $70,138	
Totals		183	$8,012,043	$508,850